# Antimicrobial Peptides: A Promising Future Alternative to Antibiotics in Aquaculture

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Intensification in aquaculture systems has brought into question the overall well-being of fish, human and the environment. The need to produce more in the culture system imposes unintentional consequences to the fish which weakens the immune system of fish and finally leads to disease outbreak. Furthermore, introduction of non-native fish species also introduces many transboundary aquatic animal diseases. Up to 40 percent of shrimp aquaculture production is lost annually, worth more than $3 billion, mainly due to viral pathogens (Stentiford *et al*. 2012). Disease losses in aquaculture can account for about 30 percent of total operating costs (Lee and O'Bryen 2003).

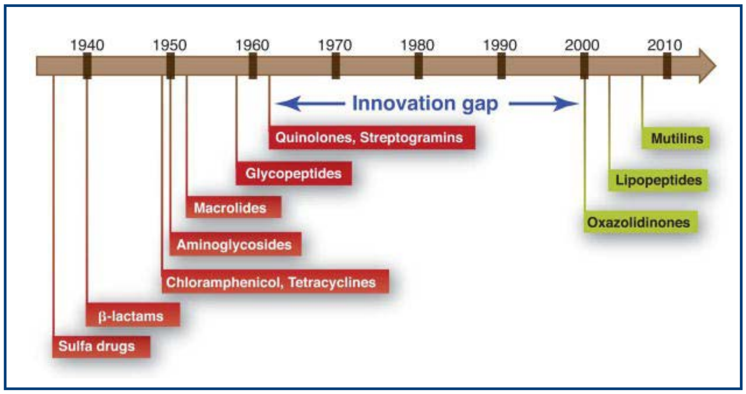


FIGURE 1. *The lack of introduction of potent new antibiotics in the latter half of last century. (Adapted from Fischbach and Walsh 2009.)*

To efficiently control disease outbreaks in aquaculture systems, a "prevention is better than treatment" approach based on the principle of prophylaxis is the method of choice. As examples, immunostimulants, probiotics, prebiotics and vaccines are being used for disease prevention. However, especially in intensive culture environments, mass mortality occurs even after taking precautions. To prevent the stock being wiped out after a disease outbreak, use of chemotherapeutics such as antibiotics, antiviral drugs and other chemical therapeutants like formaldehyde, sodium chloride, potassium permanganate are used.

The gross global investment in aquatic animal health products is $274.4 million for antibiotics and $29.4 million for antiparasitics (The Fish Site 2010). Apart from these drugs, next-generation antimicrobials and antimicrobial peptides with broad-spectrum activity are in focus as alternate therapeutic agents in aquaculture.

## Antimicrobial Resistance and Need for Novel Antimicrobial Agents in Aquaculture

Antimicrobial drugs have been the most common choice for treating many bacteria-borne diseases in people and livestock, including aquaculture, for over 50 years with tremendous benefits. But inappropriate administration of different classes of antimicrobials in aquaculture provides a selective pressure, creating a reservoir of multiple antimicrobial-resistant bacteria in cultured fish and shrimp and in the culture environment. Antimicrobial resistance is rising alarmingly. In aquaculture, antibiotic resistance has been reported in pathogenic bacteria of fish such as *Aeromonas salmonicida*, *A. hydrophila*, *Vibrio anguillarum*, *Pseudomonas fluorescens*, *Pasteurella piscicida*, *Edwardsiella tarda* (Aoki 1988) and *Yersinia ruckeri* (DeGrandis and Stevenson 1985). The presence of multiple antibiotic-resistance bacteria in aquaculture food products has become a serious human health threat because there is a potential that genes responsible for drug resistance may be transferred to bacteria in humans (Kathleen *et al*. 2016).

Although the number of multi-drug resistant bacteria is increasing, the invention of new antibiotics has been very low. From 1960s to early 2010, only four new classes of antibiotics were introduced and none have made any substantial impact (Fig. 1). The global antibiotic market is still largely dominated by antibiotics discovered before the 1960s and the few antibiotics produced since then were synthetic derivatives of existing core structures (Fischbach and Walsh 2009). In this regard, developing and finding new compounds with broad-spectrum antimicrobial activity is a pressing need. This scenario even worse in the case of aquaculture because only one antibiotic (oxytetracycline hydrochloride) is approved by the US Food and Drug Administration for use against different bacterial diseases. Hence, it is the utmost priority to find an alternative antimicrobial agent to reduce production losses from disease epizootics in aquaculture systems.

## Antimicrobial Peptides as Novel Antimicrobial Agent

Antimicrobial peptides (AMPs) are a group of highly conserved oligopeptides with five to over 100 amino acids. They are pivotal humoral components of innate immunity and have been extensively studied in invertebrates and vertebrates including fish and exhibit broad-spectrum antimicrobial activity *in vitro* and *in vivo*. They are cationic molecules that can selectively attack negatively-charged bacterial cell membranes and brings about destruction of the organism by pore formation or destabilization of membrane equilibrium or by penetration into the cell and

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thereby the impairment of cellular machinery (Reddy *et al*. 2004) (Fig. 2). Because of the wide activity against different microbial agents, these molecules have made their mark as an emerging class of natural antibiotics. Apart from having an antimicrobial role against many microorganisms, the salient features of AMPs are their broad-spectrum antimicrobial activity towards multi-drug resistant microbial isolates and very minimal chance to allow development resistance due to rapid lysis of cell membranes (Lai and Gallo 2009) (Fig. 2). Antimicrobial peptides (AMPs) have been isolated and characterized as a novel and useful alternative to antibiotics (Mookherjee and Hancock 2007). Even disease-resistant transgenic fish stock was developed to target selected AMPs (Buchanan *et al*. 2001, Sarmasik *et al*. 2002).

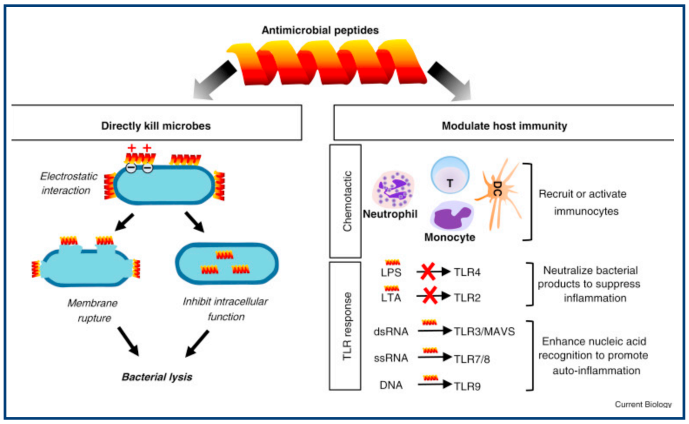


FIGURE 2. *Biological roles played by different AMPs. (Adapted from Diamond* et al. *2009.)*

## Fish Antimicrobial Peptides

Most fish AMPs are potent antibiotics with a broad spectrum of activity at micromole concentrations (Rajanbabu and Chen 2011). Although the first AMP found in fish was purified in 1995 (Pilet *et al*. 1995), it took almost a decade to learn about its role as a major component of the innate immune response.

***Piscidins*** are the family of linear, amphipathic AMPs. The first member of this family was identified from skin mucous secretions of the winter flounder *Pleuronectes americanus* and called pleurocidin (Cole *et al*. 1997). Other piscidins homologous to pleurocidins were identified in other teleosts (named asmisgurin, moronecidin, epinecidin and dicentracin). Generally, piscidins are found in gill, skin and intestine of finfish. They have potent antimicrobial activity against a variety of microorganisms. These are specifically effective against Gram-positive and -negative bacteria, with the best antimicrobial activity observed in the case of *Streptococcus*, *Pseudomonas*, *Bacillus* and *Vibrio* species. Other than bacteria, piscidins have activity against fungi (Niu *et al*. 2013), parasites (Colorni *et al*. 2008), and viruses (Wang *et al*. 2010). Piscidins have immunomodulatory capacity to strengthen the innate immune system by modulating certain immune system-related genes (Masso-Silva and Diamond 2014).

***Hepcidins*** are cysteine-rich peptides with wide availability among vertebrates including mammals, reptiles, amphibians and fish. The first hepcidin in fish was identified and isolated from hybrid striped bass (Shike *et al*. 2002) and since then hepcidins have been identified in around 40 other teleosts. In fish two forms of hepcidins can be identified: HAMP1 and HAMP2. HAMP1 is the most abundant member and present in both actinopterygian and non-actinopterygian fish but HAMP2 has been found only in actinopterygian fish. Fish hepcidins are potent antimicrobials against a wide variety of bacterial fish pathogens such as *Streptococcus iniae, Yersinia* and *Pseudomonas*, even at low micromole level (Masso-Silva and Diamond 2014). These are also effective against different fungi and viruses.

***Defensins*** is the group of cysteine-rich, cationic antimicrobial peptides found in plants, fungi, invertebrates and vertebrates. Although defensins are of three different types ($\alpha$-, $\beta$-, and $\theta$-defensins) in mammals, the defensins in fish belong to the $\beta$-defensin group. $\beta$-defensins in fish were initially identified from zebrafish, fugu and puffer fish (Zou *et al*. 2007). Since then many defensins have been identified in many other marine and freshwater fish species. Fish $\beta$-defensins are mostly abundant in skin, head kidney and spleen. The antimicrobial activity of this AMP was established against pathogenic bacteria of fish such as *A. hydrophila*, *Y. ruckeri*, *V. anguillarum* and *E. tarda* and different fish-specific viruses including Singapore grouper iridovirus (SGIV), nodavirus and viral haemorrhagic septicaemia virus (VHSV).

***Cathelicidins*** is an unusual group of AMPs with varying structure and size and defined by a conserved domain called 'cathelin.' The first member of this group in fish was identified in Atlantic hagfish *Myxine glutinosa* (Uzzell *et al*. 2003). The fish cathelicidins characterized by the cathellin domain from different fish species can be subdivided into two groups: the linear peptides and those that exhibit a characteristic disulphide bond (Masso-Silva and Diamond 2014). Fish cathelicidins have antimicrobial activity against fish pathogens such as *Y. ruckeri*, *A. salmonicida, C. albicans* and *Saprolegnia parasitica*.

## Application as Therapeutics

The therapeutic potential of AMPs was evaluated for many critical diseases in mammals and recently these were evaluated for their potential to treat microbial infections in aquaculture. Among piscidins, oral and injection administration or electro-transfer of epinecidin-1 leads to enhanced survival in zebrafish and grouper infected with *V. vulnificus* and *Streptococcus agalactiae* (Lin *et al*. 2009, Pan *et al*. 2011a, 2012a). Tilapia hepcidin reduces the bacterial count and thereby fish mortality during *V. vulnificus* infection (Pan *et al*. 2012b). Transgenic zebrafish bearing tilapia hepcidin have enhanced bacterial resistance to *V. vulnificus* and *S. agalactiae* (Pan *et al*. 2011b).

## Conclusion

Large-scale microbial infection in aquaculture frequently leads to considerable economic losses, as there are very few approved drugs available to counteract such a problem. Harnessing the potential of fish AMPs will give us a novel natural antimicrobial drug to handle this crisis situation against antibiotic-resistant microbes. More pronounced efforts are needed in the future to convert this baseline information into a potent, multifaceted, cost-effective drug for aquaculture.

## *Notes*

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